

Naval Dental Research Institute

NDRI-PR 91-07
NOVEMBER 1991

(2)



AD-A245 507



DTIC
ELECTE
JAN 27 1992
S D D

USE OF MONOCLONAL ANTIBODIES TO ENUMERATE SPIROCHETES
AND IDENTIFY TREPONEMA DENTICOLA IN DENTAL PLAQUE
OF CHILDREN, ADOLESCENTS AND YOUNG ADULTS

S. L. BARRON
G. R. RIVIERE
L. G. SIMONSON
S. A. LUKEHART
D. E. TIRA
D. W. O'NEIL

This document has been approved
for public release and sale; its
distribution is unlimited.

92-01679



Naval Medical Research and Development Command
92 1 21 009 Bethesda, Maryland

NAVAL DENTAL RESEARCH INSTITUTE
BUILDING 1-H
GREAT LAKES, ILLINOIS 60088-5259

USE OF MONOCLONAL ANTIBODIES TO ENUMERATE SPIROCHETES
AND IDENTIFY TREPONEMA DENTICOLA IN DENTAL PLAQUE
OF CHILDREN, ADOLESCENTS AND YOUNG ADULTS

S. L. BARRON
G. R. RIVIERE
L. G. SIMONSON
S. A. LUKEHART
D. E. TIRA
D. W. O'NEIL

Research Progress Report NDRI-PR 91-07
Work Unit 61102A.3M161102.BS10
Naval Medical Research and Development Command
National Naval Medical Center
Bethesda, Maryland 20889-5044

The opinions expressed herein are those of the authors and cannot be construed as reflecting the views of the Navy Department or the Naval Service at large. The use of commercially available products does not imply endorsement of these products or preference to other similar products on the market.

This document has been approved for public release; its distribution is unlimited.

Approved and released by:



D. C. CECIL III
Captain, Dental Corps
United States Navy
Commanding Officer

Use of monoclonal antibodies to enumerate spirochetes and identify *Treponema denticola* in dental plaque of children, adolescents and young adults

Barron SL, Riviere GR, Simonson LG, Lukehart SA, Tira DE, O'Neil DW. Use of monoclonal antibodies to enumerate spirochetes and identify *Treponema denticola* in dental plaque of children, adolescents and young adults. *Oral Microbiol Immunol* 1991; 6: 97-101.

Identification of spirochetes in dental plaque is difficult. Not all spirochetes can be cultured and microscopic analysis based on darkfield or phase optics cannot determine the genus and species of individual bacterial cells. The purpose of this study was to use monoclonal antibodies in an immunoenzyme technique to stain spirochetes in dental plaque. Separate mAb were used to estimate total spirochetes and relative numbers of 2 distinct types of *Treponema denticola*. Plaque samples were collected from 40 subjects grouped by age. Results showed that older subjects are more likely to have spirochetes, to have more spirochetes and to have more diverse populations of spirochetes than younger subjects. Our studies suggest that *T. denticola* may be the first treponeme to colonize the primary dentition, that *T. denticola* appears to comprise a major proportion of all spirochetes at all ages and that two distinct serotypes of *T. denticola* are found to coexist in plaque from most children.

Spirochetes can be found in dental plaque associated with apparently healthy periodontal structures of both children (1) and adults (15). However, spirochetes are found most often and in greatest numbers in association with gingival inflammation and periodontal disease in both children (1, 10, 17) and adults (6, 7, 11). These associations are based on observations derived from experiments using culture techniques (7) or microscopic analyses using darkfield (1, 2, 7, 10, 17) or phase optics (9). *In vitro* isolation and culture permit identification of genus and species but are restricted in application to those spirochetes that are isolated by chance and that will replicate in the laboratory. Culture methods underestimate the variety of spirochetes in plaque because many spirochetes observed microscopically cannot be grown *in vitro* (7, 16). Microscopic approaches permit the rapid identification of morphologic types and relative numbers of spirochetes in

plaque but cannot be used to identify either genera or species (10).

Simonson et al. recently produced monoclonal antibodies (mAb) against *Treponema denticola* (14) and showed that the amount of *T. denticola* in plaque increased directly in proportion to the severity of periodontal disease (12). This work suggested that mAb could also be used to detect and identify specific treponemes in dental plaque using conventional immunostaining methods. The purpose of this investigation was to use mAb to estimate relative numbers of spirochetes present in dental plaque and to estimate the relative numbers of discrete types of *T. denticola* within this population.

Material and methods

Subjects

Forty subjects (2-25 years, 24 male, 16 female) were included in the study. All subjects had 4 first molars (primary or

S. L. Barron¹, G. R. Riviere²,
L. G. Simonson³, S. A. Lukehart⁴,
D. E. Tira⁵, D. W. O'Neil⁶

Departments of ¹Pediatric Dentistry, ²Oral Biology, ³Behavioral Sciences and ⁴Pediatric Dentistry, School of Dentistry, University of Missouri, Kansas City, Missouri, ⁵Scientific Investigations Department, Naval Dental Research Institute, Great Lakes, Illinois, ⁶Department of Medicine, School of Medicine, University of Washington, Seattle, Washington, USA



Accession For	
NTIS	CRA&I
DTIC	TAB
Unannounced	
Justification	
By	
Distribution	
Availability Codes	
Dist	Availability Codes
A-1	20

Key words: monoclonal antibody; spirochete; *Treponema denticola*; plaque

George R. Riviere, Department of Pediatric Dentistry, School of Dentistry, Oregon Health Sciences University, Portland, OR 97201, USA

Accepted for publication June 27, 1990

permanent) and 4 maxillary and mandibular incisors (primary or permanent). No subject had periodontal disease or any systemic disease and no subject had received any systemic drug therapy, including antibiotics, within 3 months. No subject acknowledged a history of any spirochetal disease. Subjects had not received a dental prophylaxis within 6 months.

Subjects were divided into 4 groups of 10 each according to age: Group A, ages 2-4 (deciduous dentition); Group B, ages 5-11 (mixed dentition); Group C, ages 12-15 (permanent dentition, around puberty); Group D, ages 16-25 (young adults). Subjects were grouped without regard to gender since gender has not been associated with distribution of oral spirochetes in young subjects (10, 17).

Dental plaque

Plaque was collected from gingival sulci at mesiobuccal line angles of all first

Table 1. Expected reactivity of monoclonal antibodies

mAb	Source (Reference)	Reactivity
C2-1	S. Lukehart, Seattle, WA (8)	<i>Borrelia recurrentis</i> <i>Leptospira interrogans</i> <i>T. denticola</i> <i>T. hyodysenteriae</i> <i>T. pallidum</i> subsp. <i>pallidum</i> <i>T. pallidum</i> subsp. <i>pertenue</i> <i>T. phagedenis</i> (Reiter) <i>T. vincentii</i>
TD-II	L. Simonson, Great Lakes, IL (14)	<i>T. denticola</i> ATCC 33521
TD-III ¹	L. Simonsen	<i>T. denticola</i> ATCC 33520 <i>T. denticola</i> ATCC 35404
H9-1	S. Lukehart (5, 8)	<i>T. pallidum</i> subsp. <i>pallidum</i> <i>T. pallidum</i> subsp. <i>pertenue</i>
Bg	L. Simonson (13)	<i>Bacteroides gingivalis</i>

¹Unpublished observations

molars, from the facial surfaces of maxillary right central incisors, and from facial surfaces of mandibular right central incisors. The curette blade was cleaned with a new alcohol swab between each site collection. Thus, although plaque collection was standardized, in order to obtain enough plaque from young children with healthy gingiva, the 6 samples for each patient were pooled into 0.5 ml phosphate-buffered saline (PBS), pH 7.0. In order to maintain a uniform technique, all 6 sites were pooled for all subjects.

Each plaque sample was gently dispersed with a Pasteur pipette and 50 μ l of plaque suspension was placed on each of approximately 10 glass slides per subject. Slides were air-dried and stored at 0 °C.

Bacteria

Cultivable treponemes, including *T. denticola* (ATCC 33520, 33521, 35404,

35405), *Treponema pectinovorum* (ATCC 33763), *Treponema phagedenis* biotype Reiter (Dr. J. N. Miller, UCLA), *Treponema refringens* (Dr. R. George, Centers for Disease Control, Atlanta, GA), *Treponema scoliodontium* (J. N. Miller), *Treponema socranskii* (ATCC 35536), and *Treponema vincentii* (J. N. Miller), were grown in spiroplate broth (BBL Microbiology Systems, Cockeysville, MD) supplemented with 10% sterile, heat-inactivated (56 °C, 30 min) normal rabbit serum (GIBCO Laboratories, Grand Island, NY) at 34 °C. *Bacteroides gingivalis* (ATCC 33277) was grown in Wilkin's-Chalgren broth (Oxoid, Basingstoke, Hampshire, UK) supplemented with 5 μ g/ml hemin and 0.3 μ g/ml menadione. *Treponema pallidum* subsp. *pallidum* (Nichols strain) were grown in rabbits, extracted in PBS and stored at -70 °C. Bacteria were washed 3 times and resuspended to their original volumes in PBS supplemented with 0.02 M magnesium chloride (13) and 10

μ l of each suspension dried onto glass slides. Slides were stored at 0 °C.

Monoclonal antibodies

Sources and expected reactivities of each mAb used in this investigation are listed in Table 1. The specificities of each mAb have been described previously (5, 8, 13, 14). Monoclonal antibody TDII, IAA11 (serovar "B") will be referred to hereafter as TD-II; TDIII, IIBB2 (serovar C) as TD-III; and BgII, VF9/2d as Bg.

Immunocytochemistry

Dried plaque and control bacteria on slides were fixed in 100% acetone at 4 °C for 10 min, air-dried, and hydrated in PBS just before staining. Reagents were applied in 50- μ l volumes at room temperature in a humidifier and slides were rinsed and immersed in PBS for 5 min between each step except as noted. Normal rabbit serum blocking solution (10% NRS) (Zymed Laboratories, catalog #95-6561) was applied for 10 min, drained and, without washing, mAb diluted 1:2 in blocking solution was added for 60 min. Plaque sample slides for each subject were processed with mAb C2-1, H9-1, and Bg. Plaque samples reacting with C2-1 were processed with TD-II and TD-III in subsequent experiments. Prediluted biotinylated-rabbit anti-mouse antibodies (polyspecific for IgA, IgG, and IgM, H and L chains) (Zymed Laboratories, catalog #61-6440) were applied for 10 min. Streptavidin-alkaline phosphatase conjugate (1:100 dilution in PBS) (Zymed Laboratories, catalog #43-4322) was added for 10 min and slides were washed as before. Substrate (p-nitrophenyl phosphate) and chromogen (fast blue) solution (Zymed Laboratories, catalog #00-2204) was added for 15 min. Slides were washed in distilled water, blotted and coverslips applied with a glycerine-based mounting solution.

Slides were viewed at $\times 1000$ magnification. All blue-stained spirochetes in 5 non-overlapping high-power fields were counted and an average was obtained for each mAb and each subject. A mean relative number, \pm one standard deviation, of spirochetes per high-powered field stained by each mAb in each group was computed.

Controls

One plaque slide per subject per mAb analysis was treated with PBS instead

Table 2. Observed specificities of monoclonal antibodies

	C2-1	TD-II	TD-III	H9-1	Bg
<i>B. gingivalis</i>	-	-	-	-	+
<i>T. denticola</i> ATCC 33520	+	-	+	-	-
<i>T. denticola</i> ATCC 33521	+	+	-	-	-
<i>T. denticola</i> ATCC 35404	+	-	+	-	-
<i>T. denticola</i> ATCC 35405	+	-	+	-	-
<i>T. pallidum</i>	+	-	-	+	-
<i>T. pectinovorum</i>	+	+	-	-	-
<i>T. phagedenis</i>	+	-	-	-	-
<i>T. refringens</i>	+	-	-	-	-
<i>T. scoliodontium</i>	+	-	-	-	-
<i>T. socranskii</i>	+	-	-	-	-
<i>T. vincentii</i>	+	-	-	-	-

¹These cross-reactions were not observed in whole-cell ELISA (Simonson, unpublished observations).

Table 3. Mean relative number of bacteria per viewing field stained with mAb

Group (Age)	mAb			
	C2-I	TD-II	TD-III	Bg
A (2-4)	1.20 ± 2.41*	0.70 ± 0.98	1.52 ± 2.11	0.00 ± 0.00
B (5-11)	28.74 ± 38.71	4.36 ± 5.77	8.35 ± 11.44	0.36 ± 0.76
C (12-15)	9.66 ± 7.69	1.72 ± 1.45	6.00 ± 6.93	0.04 ± 0.13
D (16-25)	10.48 ± 9.47	2.14 ± 1.51	4.56 ± 3.39	0.08 ± 0.25

*Mean (± standard deviation) relative number of bacteria per viewing field stained with mAb.

of mAb as a negative (reagent) control. H9-1 was used as an additional negative (treponeme mAb) control since this mAb reacts only with a determinant only present on *T. pallidum* subspecies.

T. phagedenis biotype Reiter and *T. denticola* ATCC 33521 were used as positive (bacteria) controls for monoclonals C2-I and TD-II, respectively. The same 2 treponemes were used as negative controls when PBS was used instead of mAb on control bacteria slides.

B. gingivalis ATCC 33277 and *T. denticola* ATCC 33520 served as positive controls for monoclonals Bg and TD-III, respectively.

Statistical analysis

The Newman-Keuls multiple comparison technique for one-way analysis of variance was used to compare differences between means among the 4 age group for each mAb. Significance was set at the 0.05 level. Pearson's product-moment coefficient of correlation was used to determine relationships between relative numbers and prevalence of bacteria and age. Significance was determined with *t*-tests with $\alpha \leq 0.05$.

Results

Specificity of monoclonal antibodies

Each mAb was tested against each control bacteria (Table 2). C2-I reacted, as expected, with every treponeme tested, including *T. scrofulaceum* and *T. socranskii*, but not with *B. gingivalis*. Td-II reacted with *T. denticola* ATCC 33521, and, unexpectedly, with *T. pectinovorum*. Td-III bound *T. denticola* ATCC 33520 and *T. denticola* ATCC 35404 as predicted, but also attached to *T. denticola* ATCC 35405. The specificities of Td-II and Td-III are non-overlapping. H9-1 did not react with any oral treponeme tested.

Enumeration of spirochetes in plaque

Negative control slides were uniformly negative, demonstrating that rabbit antibodies did not crossreact with human antibodies in plaque and that neither mAb H9-1, biotin nor streptavidin was bound nonspecifically by plaque components. Negative controls also indicated that plaque spirochetes did not express endogenous alkaline phosphatase activity under these conditions.

Spirochetes were detected with C2-I in each age group (Table 3). Group B (ages 5-11 years) had the highest mean relative number of spirochetes, 28.74 ± 38.71 and Group A (2-4 years) the lowest, 1.20 ± 2.41. There was a difference ($P < 0.05$) between means of Group B and Group A but no other differences between means were significant. Relative numbers of spirochetes detected with C2-I showed a relationship with age ($r = 0.50$, $P = 0.01$; Table 5). The percentages of subjects with spirochetes per group were 50%, 70%, 70% and 90% for Groups A, B, C and D, respectively (Table 4). The prevalence of spirochetes was correlated with age ($r = 0.96$, $P = 0.05$). Relative numbers of spirochetes identified with C2-I for each subject are shown in Fig. 1A. A marked increase in relative spirochete counts can be seen at approximately 9-14 years. Three 9-year-old subjects (100, 83 and 34.8 spirochetes/field, respectively) and one 10-year-old (60/field) had more spirochetes than could be plotted on the scale in Fig. 1A.

Identification of *T. denticola*

Plaque samples stained with TD-II demonstrated the presence of treponemes sharing antigens with certain strains of *T. denticola* and with *T. pectinovorum* (Table 3). The mean relative numbers of treponemes detected with TD-II ranged from 0.70 ± 0.98 (Group A) to 4.36 ± 5.77 (Group B). There was

no significant difference between means among age groups and no relationship between relative numbers detected with TD-II and age (Table 5). *T. denticola* serovar "B" prevalences ranged from 50% in Group A to 90% in Group D (Table 4) and showed a relationship with age ($r = 0.96$, $P = 0.05$; Table 5). Fig. 1B shows the relative number of treponemes detected with TD-II in relation to age. Most subjects had treponemes reactive with TD-II in dental plaque. The 3 subjects with highest counts also had high C2-I counts.

Mean relative counts of treponemes stained with TD-III ranged from 1.52 ± 2.11 to 8.35 ± 11.44 (Groups A and B, respectively) (Table 3). Again, no significant differences between means were noted among age groups and there was no relationship between relative numbers and age (Table 5). Percentages of subjects in groups positive with TD-III ranged from 40% to 90% (Table 4) and appeared to correlate with increasing age ($r = 0.97$, $P = 0.05$; Table 5). However, Fig. 1C shows the relative number of treponemes detected with TD-III in relation to age. There appeared to be increased numbers of subjects with TD-III (+) treponemes around 9-13 years due to high numbers from 5 subjects, 2 of which also had high C2-I counts; otherwise, numbers

Table 4. Percentage of subjects per group reactive with mAb

Group	C2-I	TD-II	TD-III	Bg
A	50%	50%	40%	0%
B	70%	70%	60%	30%
C	70%	70%	60%	10%
D	90%	90%	90%	10%

Table 5. Statistical analysis of the relationship between the presence of mAb-labelled treponemes and age of subjects

Correlation	r^a	p^b
C2-I count & age ^c	0.50	0.01
TD-II count & age	0.08	NS
TD-III count & age	0.10	NS
Prevalence of stained bacteria:		
<i>T. denticola</i> ^d & age	-0.40	NS
C2-I + & age	0.96	0.05
TD-II + & age	0.96	0.05
TD-III + & age	0.97	0.05
Bg + & age	0.06	NS

^aPearson's product-moment correlation coefficient; ^b*t*-test; ^cExcluding 4 scores of Group B that were greater than mean ± 3 SD; ^dSum of subjects with bacteria stained with TD-II and/or TD-III/total number of subjects.

of TD-III (+) spirochetes appeared to be constant from young children through young adults. Thus, it is unlikely that there is a significant correlation between TD-III and age.

T. denticola (TD-II and/or TD-III) was found in plaque of every child with

spirochetes (C2-1+) and, for 4/5 children with spirochetes in Group A, *T. denticola* appeared to be the major, if not sole component of the spirochetes population defined by C2-1. After approximately 4–5 years, *T. denticola* represented approximately 40–80% of plaque spirochetes.

The mean relative numbers of bacteria reacting with Bg ranged from 0.00 ± 0.00 (Group A) to 0.36 ± 0.75 (Group B) (Table 3). There were no significant differences between mean values among age groups and no correlation was found between relative numbers and age (Table 5). The prevalences for Groups A, B, C and D, respectively, were 0%, 30%, 10% and 10% (Table 4) and showed no significant relationship with age (Table 5). Fig. 1D shows that most subjects did not appear to have reactive *B. gingivalis* in dental plaque. However, 3/4 subjects who had very high numbers of spirochetes (C2-1+) and TD-II (+) treponemes also had high counts with Bg.

Discussion

This study employed monoclonal antibodies in a sensitive immunocytochemical technique to stain spirochetes within plaque samples and to identify specific treponemes within each sample. The method permits staining of single bacterial cells and, with the appropriate mAb, identification of genus, species and serotype.

The use of mAb C2-1, previously reported to react with an antigen common to *Borrelia*, *Leptospira* and *Treponema* (8), permitted the detection of spirochetes in 70% of subjects in this study, including half of the youngest group, ages 2–4 years. Since all subjects in this study had clinically healthy gingiva, these observations support the conclusion that spirochetes are part of the normal oral flora (10, 15), even in young children.

The use of mAb C2-1 in this study also supports conclusions derived from other studies (2, 7, 9) that older children tend to have more oral spirochetes than younger children. The frequency of C2-1+ samples in our studies was equivalent or slightly higher than the prevalence reported in other studies for similarly grouped children (2, 7, 9) and these differences were greatest in younger subjects. For example, Mackler & Crawford (9) reported approximately 26% and Loesche (7) reported 40% of

children ages 3–5 had spirochetes in plaque, compared with 50% observed in this study. When subjects aged 5 through 11 were assessed with C2-1, 70% demonstrated spirochetes, while prior studies with similarly grouped ages reported 66% (2) and 50% (7). Another study reported much higher percentages than the present investigation, finding spirochetes in practically all Tanzanian children aged 6–12 (10), perhaps reflecting a geographic or cultural difference in spirochete prevalence. 70% of subjects aged 12–15 demonstrated the presence of spirochetes with C2-1 but in a study by Ashley et al. (1), 88.7% of subjects 14–15 years of age were found to have spirochetes. If 14- and 15-year-old subjects were isolated from the present study, a similar percentage would be apparent. As expected, the group of 16- to 25-year-old subjects demonstrated the highest prevalence of spirochetes (90%). Finally, it should be stressed that although this assay can detect as few as one bacterial cell on a slide, sampling error and random distribution of cells within samples prevent efforts to quantify in an absolute sense.

This is the first study to use monoclonal antibodies to determine the presence of a defined genus and species of treponeme in dental plaque of children. Half (5/10) of 2- to 4-year-old subjects had treponemes detectable with TD-II, and 4 of these also had treponemes detectable with TD-III. As these 2 mAb have non-overlapping specificities, it is evident that these young children had 1 or 2 serotypes of *T. denticola* in their dental plaque. However, there were so few spirochetes in plaque from these young children that it is only safe to say that *T. denticola* is probably the first treponeme to colonize dental plaque and that it is the predominant treponeme present in dental plaque of young children.

The proportion of children with spirochetes in plaque increased with age but, after about 5 years of age, 20–46% of the total spirochete population (detected by C2-1) did not react with *T. denticola* antibodies. Furthermore, whereas total relative numbers of spirochetes (detected with C2-1) increased in a significant relationship with age, populations of *T. denticola* did not. Therefore, these data suggest that a more diverse population of spirochetes emerges in the environment of healthy periodontal tissues as children age.

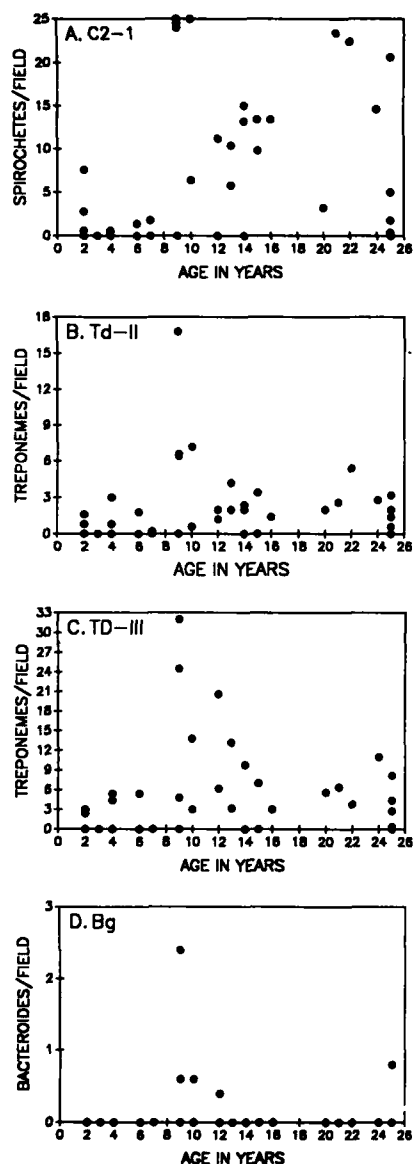


Fig. 1. Relative number of bacteria* detected with mAb.

*Scatter plot of each subject for each mAb; A = C2-1, which detects a determinant common to all spirochetes; B = TD-II, which detects *T. denticola* serovar "B"; C = TD-III, which detects *T. denticola* serovar C; and D = Bg, which detects *B. gingivalis*. Three 9-year-old and 1 10-year-old subject had off-scale counts of 100, 83, 34.8 and 60, respectively, with C2-1 and are shown at the top in Fig. 1A using contrived values of 24, 24.5 and 25. Note that scale on that y axis changes in each figure.

The prevalence and relative numbers of *B. gingivalis* were very low among the 4 age groups in this study and had no apparent relationship with age. Although *B. gingivalis* is known to be uncommon in the oral flora of children (3, 4, 17, 18), the present study provided evidence of *B. gingivalis* in dental plaque as early as 9 years of age in some subjects and in the presence of clinically healthy gingiva. These findings emphasize the utility of mAb for detecting very low numbers of bacteria in random plaque samples of children.

Acknowledgements

The investigation was supported in part by a grant from the Weldon Spring Endowment Fund (to GRR) and by a grant from the Dr. Roy James Rinehart Memorial Foundation (to SLB). The authors thank Murriel Wagoner for technical assistance and Dr. C. M. Cobb for assistance in protocol development. We also thank Professor Harris J. Keene for this thoughtful and generous contributions. This research was submitted by S. L. Barron as partial fulfillment of the requirements for a Master of Science in Oral Biology at the University of Missouri - Kansas City.

References

1. Ashley FP, Gallagher J, Wilson RF. The occurrence of *Actinobacillus actinomyces* *temcomitans*, *Bacteroides gingivalis*, *Bacteroides intermedius* and spirochetes in the subgingival microflora of adolescents and their relationship with the amount of supragingival plaque and gingivitis. *Oral Microbiol Immunol* 1988; **3**: 77-82.
2. deAraujo WC, MacDonald JB. The gingival crevice microbiota of preschool children. *J Periodontol* 1964; **35**: 285-289.
3. Delaney JE, Ratzan SK, Kornman KS. Subgingival microbiota associated with puberty: studies of pre-, circum-, and postpubertal human females. *Pediatr Dent* 1986; **8**: 275-286.
4. Frisken KW, Tagg JR, Laws AJ, Orr MB. Suspected periodontopathic microorganisms and their oral habitats in young children. *Oral Microbiol Immunol* 1987; **2**: 60-64.
5. Hook EW, Roddy RE, Lukehart SA, Hom J, Holmes KK, Tam MR. Detection of *Treponema pallidum* in lesion exudate with a pathogen-specific monoclonal antibody. *J Clin Microbiol* 1985; **22**: 241-244.
6. Listgarten MA, Helldén L. Relative distribution of bacteria at clinically healthy and periodontally diseased sites in humans. *J Clin Periodontol* 1978; **5**: 115-132.
7. Loesche WJ. The role of spirochetes in periodontal disease. *Adv Dent Res* 1988; **2**: 275-283.
8. Lukehart SA, Tam MR, Hom J, Baker-Zander SA, Holmes KK, Nowinski RC. Characterization of monoclonal antibodies to *Treponema pallidum*. *J Immunol* 1985; **134**: 585-592.
9. Mackler SB, Crawford JJ. Plaque development and gingivitis in the primary dentition. *J Periodontol* 1973; **44**: 18-24.
10. Mikx FH, Matee MI, Schaeken MJM. The prevalence of spirochetes in the subgingival microbiota of Tanzanian and Dutch children. *J Clin Periodontol* 1986; **13**: 289-293.
11. Moore WEC, Holdeman LV, Smibert RM et al. Bacteriology of experimental gingivitis in young adult humans. *Infect Immun* 1982; **38**: 651-667.
12. Simonson LG, Goodman CH, Bial JJ, Morton HE. Quantitative relationship of *Treponema denticola* to severity of periodontal disease. *Infect Immun* 1988; **56**: 726-728.
13. Simonson LG, Merrell BR, Rouse RF, Shklair IL. Production and characterization of monoclonal antibodies to *Bacteroides gingivalis*. *J Dent Res* 1986; **65**: 95-97.
14. Simonson LG, Rouse RF, Bockowski SW. Monoclonal antibodies that recognize a specific surface antigen of *Treponema denticola*. *Infect Immun* 1988; **56**: 60-63.
15. Socransky SS, Gibbons RJ, Dale AC, Bortnick L, Rosenthal E, MacDonald JB. The microbiota of the gingival crevice area of man. I. Total microscopic and viable counts of specific organisms. *Arch Oral Biol* 1963; **8**: 275-280.
16. Thornburg RW, Baseman JB. Comparison of major protein antigens and protein profiles of *Treponema pallidum* and *Treponema pertenue*. *Infect Immun* 1983; **42**: 623-627.
17. van Oosten MAC, Mombelli A, Gusberti FA, Lang NP. Black-pigmented *Bacteroides* and spirochetes in the subgingival microbiota of prepubertal schoolchildren. *J Periodont Res* 1988; **23**: 199-203.
18. Yanover L, Ellen RP. A clinical and microbiologic examination of gingival disease in parapubescent females. *J Periodontol* 1986; **57**: 562-567.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) USE OF MONOCLONAL ANTIBODIES TO ENUMERATE SPIROCHETES AND IDENTIFY <u>TREPONEMA DENTICOLA</u> IN DENTAL PLAQUE OF CHILDREN, ADOLESCENTS AND YOUNG ADULTS		5. TYPE OF REPORT & PERIOD COVERED
7. AUTHOR(s) S. L. BARRON, G.R. RIVIERE, L. G. SIMONSON, S. A. LUKEHART, D. E. TIRA, D. W. O'NEIL		6. PERFORMING ORG. REPORT NUMBER NDRI-PR 91-07
9. PERFORMING ORGANIZATION NAME AND ADDRESS Naval Dental Research Institute Building 1-H Great Lakes, IL 60088-5259		8. CONTRACT OR GRANT NUMBER(s)
11. CONTROLLING OFFICE NAME AND ADDRESS Naval Medical Research and Development Command, National Naval Medical Center, Bethesda, MD 20889-5259		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 61102A.3M161102.BS10 ✓
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) Chief, Bureau of Medicine and Surgery Navy Department Washington, D.C. 20372-5120		12. REPORT DATE November 1991
		13. NUMBER OF PAGES (5)
		15. SECURITY CLASS. (of this report) UNCLASSIFIED
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) This document has been approved for public release; distribution unlimited.		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report) This document has been approved for public release; distribution unlimited.		
18. SUPPLEMENTARY NOTES Oral Microbiol Immunol 1991: 6: 97-101		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Monoclonal Antibody; Spirochetes; <u>Treponema denticola</u> ; Plaque		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Identification of spirochetes in dental plaque is difficult. Not all spirochetes can be cultured and microscopic analysis based on darkfield or phase optics cannot determine the genus and species of individual bacterial cells. The purpose of this study was to use monoclonal antibodies in an immunoenzyme technique to stain spirochetes in dental plaque. Separate mAb were used to estimate total spirochetes and relative numbers of 2 distinct types of <u>Treponema denticola</u> . Plaque samples were collected		

DD FORM 1 JAN 73 1473

EDITION OF 1 NOV 68 IS OBSOLETE

S/N 0102-LF-014-6601

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

from 40 subjects grouped by age. Results showed that older subjects are more likely to have spirochetes, to have more spirochetes and to have more diverse populations of spirochetes than younger subjects. Our studies suggest that T. denticola may be the first treponeme to colonize the primary dentition, that T. denticola appears to comprise a major proportion of all spirochetes at all ages and that two distinct serotypes of T. denticola are found to coexist in plaque from most children.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE(When Data Entered)